Research Article

Received: 1 August 2010 Revised: 17 November 2010 Accepted: 19 November 2010 Published online in Wiley Online Library: 11 January 2011

(wileyonlinelibrary.com) DOI 10.1002/jsfa.4268

Survey of *ex situ* fruit and leaf volatiles from several *Pistacia* cultivars grown in California[†]

James N Roitman,[‡] Glory B Merrill[‡] and John J Beck*

Abstract

BACKGROUND: California is the second largest cultivator of pistachios, producing over 375 million pounds and a revenue of \$787 million in 2009. Despite the agricultural and economic importance of pistachios, little is known regarding their actual volatile emissions, which are of interest owing to their potential roles as semiochemicals to insect pests.

RESULTS: The *ex situ* volatile analysis of leaves from *Pistacia atlantica, P. chinensis, P. lentiscus, P. palaestina, P. terebinthus, P. vera* and *P. weimannifolia* demonstrated emission differences between species as well as between female and male leaves. Leaves from the female *P. vera* cultivars Bronte, Damghan, II, III, Kerman and Ohadi as well as fruits of *P. atlantica, P. chinensis, P. lentiscus, P. palaestina, P. terebinthus* and *P. vera* (cultivars II, III, Kaleh, Kerman, Momtaz and Ohadi) showed differences in the composition and relative quantity of major volatiles. The compounds in highest relative quantities from the various analyses were sabinene, Δ^3-carene, β-myrcene, α-phellandrene, limonene, (*Z*)-ocimene, (*E*)-β-ocimene and α-terpinolene.

CONCLUSION: This is the first *ex situ* survey of fruit and leaf volatile emissions from California-grown *Pistacia* species and a number of corresponding cultivars. The study provides an overview of the major and minor volatile emissions and also offers evidence of chemotypes based on monoterpenes. The results highlight the dissimilarity of major components detected between *ex situ* volatile collection and essential oil analysis.

Keywords: cultivars; *ex situ*; fruits; leaves; limonene; ocimene; *Pistacia*; volatiles

INTRODUCTION

Trees of the genus *Pistacia* (Anacardiaceae), commonly known as pistachio, are an important agricultural commodity of California, which produced over 375 million pounds in 2009 with associated revenues of \$787 million.[1] California pistachio crops primarily comprise the *P. vera* L. single female cultivar Kerman and the male pollinator 'Peters', which together produce more than 90% of the US supply of pistachios. Researchers have recently reported investigations into expanding the number of cultivars for use by the pistachio industry for production and marketing reasons.[2] Additionally, dependence on a single cultivar for crop production has raised concerns regarding crop vulnerability to insect pests and diseases.[3]

Because of the dependence on a single pistachio cultivar in California, little is known regarding the volatile emissions of other *Pistacia* species and their related cultivars. Iran is the major cultivator of pistachios in the world, producing more than 500 million pounds in 2008;[4] thus the majority of research on the chemical composition of *Pistacia* species has been performed on plants in central/western Asia, the origin of *P. vera*, as well as neighbouring countries. Most of these reports are on the essential oil (EO) content and not the actual volatile emission patterns.[5–8] Furthermore, geographical differences are known to influence the quantity and composition of secondary metabolites, so these reports may not directly translate to California-grown pistachios.[9]

Pistacia vera cv. Kerman has been identified as a major emitter of monoterpenes in California's Central Valley,[10] and EOs have been found to correlate with monoterpene emissions.[11] A recent study of California 'Kerman' EO composition and quantity did show a correlation between EO content and collected *in situ* monoterpene emission, though it did not compare relative quantities of similar volatiles.[12]

The collection, identification and implementation of volatiles from agricultural crops for use as semiochemicals are priorities of the tree nut industry. Insect pests have been shown to use cues from plant-derived monoterpenes, sesquiterpenes and green leaf volatiles (GLVs).[13] These chemical cues, semiochemicals, can modulate insect behaviours such as attraction, acceptance or rejection of a plant part for feeding, ovipositional activity and/or larval development. Recently, volatiles collected from almonds have been investigated as semiochemicals of *Amyelois transitella*

* Correspondence to: John J Beck, USDA-ARS, WRRC, Plant Mycotoxin Research, 800 Buchanan street, Albany, CA 94710, USA. E-mail: john.beck@ars.usda.gov

‡ Retired.

United States Department of Agriculture, Agricultural Research Service, Western Regional Research Center, Plant Mycotoxin Research, 800 Buchanan Street, Albany, CA 94710, USA

(Lepidoptera: Pyralidae), commonly known as navel orangeworm (NOW), an insect pest to pistachio and almond orchards.[14,15]

As part of our ongoing research efforts to identify plant-derived semiochemicals for insect pests of the California tree nut industry, the *ex situ* volatile emission of leaves and fruits from several *Pistacia* species grown at the USDA National Clonal Germplasm Repository (Davis, CA, USA) was investigated. The results of the investigation enabled comparison of the major and minor volatile output of *Pistacia* within and among species. While the qualitative compositions of the present *ex situ* volatile study and reported EO analyses were highly corroborative, the relative quantities, particularly among the major amounts detected, displayed a large degree of disparity. Finally, the major components of the seven *Pistacia* species and the eight varieties provide insight into the chemotaxonomy of *Pistacia* and highlight the need for further investigation into this area of research.

MATERIALS AND METHODS

Plant material

Samples were collected between June and August 2003 from the orchards of the USDA National Clonal Germplasm Repository for Fruit and Nut Crops (Winters, CA, USA). For leaves, branches approximately 3–4 feet in length were removed from the trees and immediately placed in water. For fruits, clusters were removed and placed in paper bags. Intact fruits and leaves were removed, segregated and placed in collection chambers. Volatile collections were performed within 1 day of sample acquisitions.

Volatile collection

Volatile analyses were performed similarly to previously reported methods.[16] Briefly, 800 g samples of plant material were placed in 12 L round-bottomed flasks, which were then sealed with aluminium caps fitted with inlets for air flow and Teflon gaskets. Purified air was passed through the samples at 1 L min^{-1} and volatiles were collected onto Tenax (10 g) for 18 h. The volatiles were desorbed using freshly distilled diethyl ether (40 mL), then concentrated to a volume of *ca* 1 mL using a warm water bath and a Vigreux distillation column.

Gas chromatography/mass spectrometry (GC/MS) analysis of collected volatiles

Separation and identification of the collected volatiles were achieved via standard methods typically utilised by this laboratory.[12,14] Instrumentation was as follows: a J&W Scientific (Folsom, CA, USA) DB-Wax column (60 m × 0.32 mm i.d. × 0.25 μm) or a J&W Scientific DB-1 column (60 m × 0.32 mm i.d. × 0.25 μm) installed on one of two HP-6890 gas chromatographs (GC) coupled to HP-5973 mass-selective detectors (MS) (Hewlett Packard, Palo Alto, CA, USA). Desorbed volatiles were analysed with the following methods. For DB-Wax: injector temperature, 200 °C; split mode (10 : 1), split flow, 19.8 mL min^{-1}; inlet temperature, 200 °C; constant flow, 3 mL min^{-1}; oven settings, initial temperature, 40 °C; hold time, 0 min; ramp one, 4 °C min^{-1}; final temperature, 200 °C; hold time, 40 min. For DB-1: injector temperature, 200 °C; split mode (10 : 1), split flow, 19.8 mL min^{-1}; inlet temperature, 200 °C; constant flow, 2 mL min^{-1}; oven settings, initial temperature, 40 °C; hold time, 0 min; ramp one, 4 °C min^{-1}; final temperature, 250 °C; hold time, 30 min. Mass-selective detector parameters: source temperature, 230 °C; MS source temperature, 150 °C; electron ionisation mode, 70 eV; solvent delay, 1 min; scan group 1, 40–300 amu; scan group 2 at 20 min, 40–450 amu. NIST, Wiley and internally generated databases were used for fragmentation pattern identification. Retention indices (RIs) were calculated using a homologous series of *n*-alkanes on the DB-Wax and DB-1 columns. Unless noted otherwise, each experiment was performed in duplicate.

Calculated RIs were used for compound identification and comparison of DB-1 and DB-Wax column results. The relative abundances (peak areas) were noted and the inclusion of a volatile in Tables 1–4 was based on its presence in both GC analyses as well as it having a minimum peak area of 0.5% of the largest volatile organic compound peak present in each run. The averages of the relative peak areas from each experiment were converted to the corresponding percentages of the total peak areas. One of the reviewers correctly pointed out that there may be errors in quantitation owing to the lack of proportionality between peak areas and concentrations as obtained by GC/MS. However, the results do identify the various volatile compounds involved and provide a starting point for future investigations involving *Pistacia* species. Data analyses (means, counts and graphing) were performed with Microsoft Excel software (Redmond, WA, USA).

RESULTS AND DISCUSSION

The *ex situ* collection and analysis of the fruits and leaves and female and male cultivars from seven *Pistacia* species provided a total of 50 volatiles ranging from major to minor amounts. Table 1 shows the results of the analysis of the seven cultivars for leaves only and allows for the comparison of female and male volatile emissions. At first glance the most noticeable result is the relatively larger presence of monoterpenes, generally in the calculated DB-1 RI range 930–1077 (Table 1), compared with the relatively sparse number of volatiles made up of GLVs, sesquiterpenes, aromatics, esters and related compounds. This observation is of particular interest when compared with the volatile emission profiles of almonds, which along with pistachios are the primary host plants of the insect pest NOW.

For *ex situ* almond emissions[16] the number of monoterpenes to sesquiterpenes is nearly equal, albeit both in relatively low number of occurrences. For *in situ* almond volatile analyses[14] the number of monoterpenes is lower and is vastly outnumbered by sesquiterpenes 7 : 1. Compare this relative ratio with the current *ex situ* pistachio volatile study and the total occurrences of 19 monoterpenes to 14 sesquiterpenes; however, for the species listed in Table 1, the comparative total amounts are *ca* 18 : 1 monoterpenes to sesquiterpenes, based on the integration of relative peak areas. These estimated differences between terpenoid emissions from pistachios appear to be mirrored in the diverse number of reports regarding the EO content of various *Pistacia* species.[6,12,17–20] However, ongoing research of the actual ambient volatile emission patterns and differences between pistachio and almond may soon provide researchers with more evidence of the native volatile bouquets that insect pests are encountering during host finding.

Sesquiterpenes from leaves

Analysis of the data in Table 1 for sesquiterpenes gave the following numbers (total relative percentage of sesquiterpenes to total number of occurrences of sesquiterpenes) for the top five sesquiterpene-producing plants, in descending percentage order: female *P. terebinthus*, 19.1 : 9; female *P. lentiscus*, 17.6 : 14; male

Table 1. *Ex situ* volatile emission of leaves from female (F) *Pistacia* cultivars and male (M) pollinator *Pistacia* species[a]

	DB-1		DB-Wax			*P. atlantica*		*P. chinensis*[b]		*P. lentiscus*[b]		*P. palaestina*		*P. terebinthus*		*P. vera* cv. Kerman		*P. weimannifolia*[c]	
#	C	L	C	L	Identity	F	M	F	M	F	M	F	M	F	M	F	M	F	M
1	930	929	1020	1020	α-Pinene	5.7	9.9	1.0	0.5	7.2	6.6	0.5	14.6	9.8	5.9	0.6	6.2	3.3	3.1
2	922	922	1024	1022	α-Thujene	1.2				1.0									
3	942	941	1063	1063	Camphene	1.0	1.9				0.3			1.1	0.6		1.2	0.3	0.3
4	968	968	1106	1106	β-Pinene	2.5	4.0			2.7	3.4		2.5	5.3	1.9		0.3	0.9	0.8
5	964	964	1117	1117	Sabinene	27.4	2.5			**16.5**	2.1	0.8		2.2	1.0			0.6	0.6
6	995		1130		Unidentified monoterpene								0.2				1.3		
7	1004	1004	1143	1144	Δ^3-Carene			5.0	17.3			0.4	1.7			1.3	9.3		
8	981	981	1155	1157	β-Myrcene	5.9	4.6	2.5[d]	**60.4**	7.3[d]	4.5	5.1	4.4	10.3	**35.7**	0.7	1.2	5.5	4.7
9	996	996	1160	1160	α-Phellandrene		0.3		0.9		4.4	8.4	2.3	0.3	0.2		0.6	**38.9**	**40.8**
10	1006	1006	1177	1176	α-Terpinene	1.6			0.5	4.1		0.5	1.1				4.2		
11	1020	1020	1197	1197	Limonene	1.9	2.4	0.7	2.5	10.9	**58.6**	**65.4**	**43.9**	2.4	1.1	**53.6**	6.7	21.6	17.5
12	1018	1018	1205	1205	β-Phellandrene	1.2	0.9	0.4	1.5	11.4	8.6	1.5	1.4	1.5	0.7		0.9	16.5	21.0
13	1026	1026	1234	1229	(*Z*)-Ocimene	1.0	1.6	**45.5**	1.1	2.5	0.8	0.5	1.1	2.7	3.8	0.7	0.1	0.3	0.2
14	1048	1048	1245	1241	γ-Terpinene	2.5	0.2			6.7	0.3		1.0				1.3	0.2	0.2
15	1038	1037	1249	1245	(*E*)-β-Ocimene	**36.2**	**53.6**	**38.1**	0.6	7.4	0.7	3.9	1.1	**33.3**	**36.1**	29.8	3.4	6.9	6.9
16	1010	1010	1267	1264	*p*-Cymene	0.2				1.5	0.4	0.4	0.3			0.3	0.9	2.4	2.3
17	1077	1077	1281	1278	α-Terpinolene	0.5		0.8	5.5	1.5	0.1	7.1	12.9	0.3	0.2	5.7	**57.1**	0.6	0.6
18	1106	1105	1305	1302	(*E*)-4,8-dimethyl-1,3,7-nonatriene	6.1	10.2	0.9	0.6	0.6	0.6	0.3	0.3	8.7	0.6	1.0	0.4	0.9	0.9
19	988	986	1316	1312	(*Z*)-3-Hexenyl acetate		0.4			0.1			0.4	1.0	1.9				
20	840	834	1386	1381	(*Z*)-3-Hexen-1-ol										0.2				
21	1345	1347	1457	1456	α-Cubebene	0.5				0.2		0.2		0.6					
22	1169	1167	1461	1458	(*Z*)-3-Hexenyl butanoate					0.1				0.2					
23	1054	1051	1467	1465	Sabinene hydrate														
24	1371	1370	1482	1481	α-Ylangene			0.7						0.3					
25	1373	1374	1490	1490	α-Copaene/unidentified sesquiterpene			0.3		1.0				0.2					
26	1385	1385	1537	1536	β-Cubebene			2.9		0.1				0.3					
27	1083	1083	1549	1546	Linalool	0.6	0.4	0.6						0.4	0.6		0.3	0.3	0.2
28	1267	1268	1579	1578	Bornyl acetate	0.3	0.3	0.6									0.9		
29	1433	1434	1588	1587	α-Guaiene			0.3											
30	1411	1415	1595	1594	β-Caryophyllene				7.0	1.2	3.4	1.2	9.5	7.6	0.8			0.2	
31	1271	1273	1596	1597	2-Undecanone						0.1								
32	1159	1159	1600	1600	Terpineol-4					0.5									
33	1437	1436	1604	1605	Aromadendrene					0.6							0.1		
34	1066	1066	1617	1616	Methyl benzoate		0.4							0.6	2.1	5.8	3.4		
35	1440		1630		Unidentified sesquiterpene														

Table 1. *(Continued)*

	DB-1		DB-Wax			*P. atlantica*		*P. chinensis*[b]		*P. lentiscus*[b]		*P. palaestina*		*P. terebinthus*		*P. vera* cv. Kerman		*P. weimannifolia*[c]	
#	C	L	C	L	Identity	F	M	F	M	F	M	F	M	F	M	F	M	F	M
36	1456	1456	1643	1643	Alloaromadendrene														
37	1446	1449	1666	1666	α-Humulene/*t*-β-farnesene				0.5	0.6	0.4	0.4	1.2	0.3					
38	1467	1469	1686	1685	γ-Muurolene					1.1									
39	1329	1331	1694	1694	α-Terpinyl acetate/ledene														
40	1471	1474	1706	1707	Germacrene D	2.5	5.1			9.8	4.4	3.2		2.9	0.4			0.2	
41	1494	1492	1712	1712	α-Amorphene					0.2									
42	1476	1480	1718	1716	Valencene/β-selinene				0.4	0.1									
43	1486	1489	1721	1721	α-Selinene/α-muurolene				0.7	0.5									
44	1489	1489	1731	1732	Bicyclogermacrene	0.5	0.2			0.1				1.1	0.2				
45	1492	1496	1747	1744	(*E*, *E*)-α-farnesene	0.5	0.7			0.3				5.8	5.3			0.5	
46	1510	1514	1756	1752	δ-Cadinene							0.1							
47	1500	1505	1759	1757	γ-Cadinene					1.8									
48	1165	1166	2118	2125	Methyl salicylate									0.3					
49	1542	1542	2118	2125	(*Z*)-3-Hexenyl benzoate		0.4							0.4	0.6				
50	1252	1252	2432	2448	1*H*-Indole											0.5	0.1		

[a] Values are percentage of total relative peak areas for each run; only peaks >0.5% of largest peak are included; highest relative amount of volatile emitted is shown in bold; two bold values in a column can be considered as nearly equal (not statistically proven); unless noted otherwise, values are average of DB-1 and DB-Wax injections; compound identification by RI relative to *n*-alkanes on DB-1 and DB-Wax columns, retention times, mass fragment libraries and comparison with authentic samples; C, calculated value; L, literature value.
[b] DB-Wax only.
[c] DB-1 only.
[d] Unable to resolve compounds **8** and **9**.

P. chinensis, 8.6 : 4; male *P. terebinthus*, 6.7 : 4; female *P. palaestina*, 5.1 : 5. The top three sesquiterpenes produced over the series (both female and male) were β-caryophyllene, germacrene D and (E, E)-α-farnesene, in that order. Interestingly, the male *P. chinensis* leaves were primarily comprised of β-caryophyllene and did not contain any of the other two noted common sesquiterpenes. This result is consistent with a report on EOs of *P. chinensis* from five locales in China; however, in that report the major sesquiterpene noted is caryophyllene – distinguished from β-caryophyllene by a different RI.[18] The female leaves of *P. lentiscus* and *P. terebinthus* exhibited a relatively large occurrence of sesquiterpenes overall; both had the top three sesquiterpenes in comparatively modest amounts, and both with the females possessing a larger number of sesquiterpenes than their male counterparts – 14 to 3 for *P. lentiscus* and 9 to 4 for *P. terebinthus*. A study of EOs of *P. lentiscus* from different origins in Italy corroborates the relatively large amounts of β-caryophyllene and germacrene D; however, the major amount between the two sesquiterpenes was dependent on geographical origin.[19]

Monoterpenes from leaves

Next, attention is given to the remaining volatile components from leaves (Table 1) and comparison of the differences between species and male and female leaves. The top two major components for *P. atlantica* female leaves are (E)-β-ocimene followed by sabinene, and for male leaves are (E)-β-ocimene followed by (E)-4,8-dimethyl-1,3,5-nonatriene and α-pinene essentially tied for distant second. These major components are surprising given the detailed report on *P. atlantica* male and female leaves collected from Algiers,[17] which showed that the top two components from female leaves were Δ^3-carene with α-terpinyl acetate in distant second, and for male leaves were an α-pinene/α-thujene mix as top monoterpene followed by spathulenol. It is not known whether the chemical composition disparities are due to geographical differences or method of volatile collection (essential oil *versus ex situ*); however, the results from a study of female and male leaves from Greece[21] show different major volatiles from *P. atlantica* than those noted above. This is suggestive of geography playing an important role in *ex situ* volatile emissions, though not conclusive.

The cultivar *P. chinensis* provided the top two monoterpenes (Z)-ocimene and (E)-β-ocimene in relatively large amounts from the female, and β-myrcene and Δ^3-carene from the male. This is in contrast to the EOs reported from *P. chinensis* grown in different locales in China,[18] which showed (Z)-ocimene as the main constituent for one location but other varying major volatiles for the other locales: camphene, α-pinene, β-phellandrene and β-pinene.

Comparisons of *P. lentiscus* female and male leaf emissions show both with diverse volatile production. The female leaves have more sesquiterpenes than the male leaves and near equal monoterpene occurrences; however, the male produced significantly more of its main component, limonene, than the female's sabinene. There are reports of the EO from *P. lentiscus* grown in Greece[22] and Italy[19] that showed similarities, with α-pinene, β-myrcene and terpinen-4-ol as the main components, depending on the time of season. These volatiles are for the female, though, and thus dissimilar to the findings of the main *ex situ* volatiles of the same species grown in California.

Limonene was the main *ex situ* constituent for both male and female *P. palaestina* leaves, in contrast to the EO content of *P. palaestina* leaves and fruits grown in Jordan,[6] which showed the main constituents α-pinene in leaves and (E)-β-ocimene in fruits.

The monoterpene volatile emission from *P. terebinthus* was very similar between female and male leaves, with the main constituents being (E)-β-ocimene as the highest emission, and both emitting a total of 12 monoterpenes. The biggest difference between female and male was in relative amounts of β-myrcene – female 10.3% and male 35.7%. A report on the EO from leaves of *P. terebinthus* grown in Turkey shows terpinen-4-ol as the main monoterpene.[5]

Perhaps the best opportunity for direct comparison of *ex situ* volatile emission with that of EO content from the same species, *P. vera* cv. Kerman, and grown in the same region, the Sacramento Valley of California, is with a recent study by Dragull *et al.*[12] on female fruits, peduncles and leaves. The main EO constituents for leaves were limonene, in concurrence with the present *ex situ* leaf emissions, followed by α-terpinolene and α-pinene, which was not corroborated by the present *ex situ* study that showed (E)-β-ocimene in a strong second and α-terpinolene in a distant third. This comparison suggests a difference does exist between collection methods and that EO content is not directly related to monoterpene emission. However, as noted earlier, a conclusive assessment would be better addressed by comparison of EO content with actual ambient volatile emission of a pistachio orchard. The *ex situ* emission for male leaves was vastly different when compared with its female counterpart, with α-terpinolene as the major component followed by Δ^3-carene as the number two emission and limonene as the number three emission. The most notable absence in the *P. vera* cv. Kerman leaf emissions is that of sesquiterpenes from the female. The male leaves did show a very minor amount of aromadendrene.

Finally, the *ex situ* volatile collection from *P. weimannifolia* appears to be the first report on this species. Most notable is the high concurrence of volatile emission between female and male leaves. The only difference is the female leaves showing the three main sesquiterpenes, discussed earlier in the section above, whereas the male counterpart does not emit any sesquiterpenes. Both the female and male emitted α-phellandrene in the highest amount followed by limonene, β-phellandrene and (E)-β-ocimene – but in differing order of amounts emitted.

Table 2 provides a quick synopsis of the sum of the major volatiles emitted by female and male leaves and is sorted by both emission total relative percentages and number of occurrences of the volatiles. Figure 1 shows the chemical structures of corresponding major volatiles. An interesting result was the number of volatiles that occur in the leaves of all seven species and in both female and male: α-pinene, β-myrcene, limonene, (Z)-ocimene, (E)-β-ocimene and (E)-4,8-dimethyl-1,3,7-nonatriene. The volatiles limonene and (E)-β-ocimene were the most common to both female and male. A remarkable result of Table 2 was the disparity of the overall emission of some of the volatiles. For instance, (Z)-β-ocimene and sabinene were in relatively high abundance for the female leaves but low for male leaves. In contrast, the Δ^3-carene content in female leaves was low compared with a relatively higher amount in the male leaves There were seven out of 14 major volatiles that were consistent (occurring in all seven species) in female, and eight out of 14 consistent volatiles in male.

Volatiles from female *P. vera* cultivar leaves

To determine the differences between cultivars of a single species, the analysis of *ex situ* volatile emissions of leaves from various female *P. vera* cultivars was performed. The major volatiles are shown in Table 3 and the 'Kerman' emissions are

Table 2. Sum of major volatile amounts and occurrences from female (F) and male (M) leaves[a]

	Sorted by female volatiles					Sorted by male volatiles			
	Totals		Occurrences			Totals		Occurrences	
Identity	F	M	F	M	Identity	F	M	F	M
Limonene	156.5	132.7	7	7	Limonene	156.5	132.7	7	7
(*E*)-β-Ocimene	155.6	102.5	7	7	β-Myrcene	37.3	115.5	7	7
(*Z*)-Ocimene	53.1	8.7	7	7	(*E*)-β-Ocimene	155.6	102.5	7	7
Sabinene	47.6	6.1	5	4	α-Terpinolene	16.5	76.5	7	6
α-Phellandrene	47.6	49.4	3	7	α-Phellandrene	47.6	49.4	3	7
β-Myrcene	37.3	115.5	7	7	α-Pinene	28.1	46.7	7	7
β-Phellandrene	32.6	35.1	6	7	β-Phellandrene	32.6	35.1	6	7
α-Pinene	28.1	46.7	7	7	Δ^3-Carene	6.7	28.3	3	3
(*E*)-4,8-Dimethyl-1,3,7-nonatriene	18.5	13.7	7	7	β-Caryophyllene	10.2	20.8	4	4
Germacrene D	18.5	9.9	5	3	(*E*)-4,8-Dimethyl-1,3,7-nonatriene	18.5	13.7	7	7
α-Terpinolene	16.5	76.5	7	6	β-Pinene	11.4	13.0	4	6
β-Pinene	11.4	13.0	4	6	Germacrene D	18.5	9.9	5	3
β-Caryophyllene	10.2	20.8	4	4	(*Z*)-Ocimene	53.1	8.7	7	7
Δ^3-Carene	6.7	28.3	3	3	Sabinene	47.6	6.1	5	4

[a] Volatile amounts are the sum of the total relative percentages and occurrences are out of seven plants; data correspond to material in Table 1.

Table 3. Major volatiles emitted from leaves of female *Pistacia vera* cultivars and male pollinator 'Peters'[a]

		P. vera cultivars						
#	Identity	Bronte[b]	Damghan	II	III	Kerman	Ohadi	Peters[b]
1	α-Pinene		3.8					4.8
7	Δ^3-Carene	6.8	5.7		2.0		2.0	6.6
8	β-Myrcene	4.2[c]	2.0	6.6				2.0
10	α-Terpinene	5.6	2.3					3.1
11	Limonene	9.5	4.1	**54.1**	**72.6**	**53.6**	**28.8**	4.5
12	β-Phellandrene				4.2			
13	(*Z*)-Ocimene						2.9	
14	γ-Terpinene	2.2						
15	(*E*)-β-Ocimene	2.4	**36.1**	27.9	5.7	29.8	**34.3**	14.4
17	α-Terpinolene	**55.7**	**37.2**	6.4	4.5	5.7	11.5	**47.3**
33	Aromadendrene				2.5			
34	Methyl benzoate	3.7				5.8	14.1	4.7

[a] Values are percentage of total relative peak areas for each run; only peaks >2% of total relative peak area are included; highest relative amount of volatile emitted per cultivar is shown in bold; two bold values in a column can be considered as nearly equal (not statistically proven); unless noted otherwise, values are average of DB-1 and DB-Wax injections.
[b] DB-Wax only.
[c] Unable to resolve compounds **8** and **9**.

shown for comparison. All cultivars analysed did not show any major sesquiterpene emission; however, there was the presence of methyl benzoate in four of the seven cultivars analysed. Methyl benzoate, a common volatile of numerous plants,[23] has demonstrated semiochemical behaviour and has been investigated as a minor component of a mixture of host plant volatiles that elicit a response from the oriental fruit moth (Lepidoptera).[24] Surprisingly, 'Kerman' shows the least number of monoterpenes, but it is similar to the *P. vera* cultivars II and III in the major components emitted. The *P. vera* cultivars Bronte and Damghan, female leaves, and *P. vera* cv. Kerman, male leaves, are the only plants in the present study to emit α-terpinolene as the major component. α-Terpinolene, which is often listed among the top components of EO compositions in the genus, has been reported as the major EO component in a study of *P. vera* fruits.[25]

Volatiles from fruits

Finally, Table 4 provides some insight into the *ex situ* volatile emissions of whole fruits from six *Pistacia* species. The most notable result is the similarity of the major constituent emitted by the six *P. vera* cultivars, *P. palaestina* and *P. terebinthus*, namely limonene. This phenomenon will be discussed in greater detail momentarily. It should be noted that α-phellandrene is essentially equal in amount to limonene in *P. terebinthus*. α-Phellandrene is structurally similar (Fig. 1) to limonene and also shares the same biosynthetic pathway (Fig. 2) as limonene.

Table 4. *Ex situ* volatile emission of fruits from *Pistacia* species and *Pistacia vera* cultivars[a]

#	Identity	*P. atlantica*	*P. chinensis*	*P. lentiscus*[b]	*P. palaestina*	*P terebinthus*	*P. vera* cultivars: II	III	Kaleh[c]	Kerman	Momtaz[c]	Ohadi
1	α-Pinene	4.5	1.4	5.5		8.3		2.8			3.0	3.6
2	α-Thujene	2.2					10.0	13.3	11.2	5.4	13.1	23.3
4	β-Pinene	2.0				8.1						
5	Sabinene	**46.1**		**58.5**	6.4	5.3						
7	Δ^3-Carene		**40.2**									
8	β-Myrcene	30.4	20.8	26.9	9.6	2.7	20.7	3.6	19.6[d]	2.5	3.7[d]	3.9
9	α-Phellandrene		6.6		11.4	**17.5**						
11	Limonene	5.7	5.6	3.8	**69.5**	**17.8**	**61.8**	**68.5**	**60.3**	**67.0**	**67.3**	**53.9**
12	β-Phellandrene	3.7	4.7		3.1	13.8						
15	(*E*)-β-Ocimene		3.7	2.7		2.2				13.5		
17	α-Terpinolene		9.5			3.7		5.4	8.9	11.6	10.8	15.3
18	(*E*)-4,8-Dimethyl-1,3,7-nonatriene		1.3									
21	α-Cubebene					3.1						
24	α-Ylangene					0.9						
26	β-Cubebene					2.1						
27	Linalool						7.5	4.4				
28	Bornyl acetate	3.3		2.5		1.8		2.0			2.2	
30	β-Caryophyllene		6.2			1.3						
35	Unidentified sesquiterpene					0.9						
39	α-Terpinyl acetate/ledene					0.9						
40	Germacrene D	2.1				6.4						
44	Bicyclogermacrene					3.1						

[a] Values are percentage of total relative peak areas for each run; only peaks >0.5% of largest peak are included; highest relative amount of volatile emitted is shown in bold; two bold values in a column can be considered as nearly equal (not statistically proven); unless noted otherwise, values are average of DB-1 and DB-Wax injections.
[b] DB-1 only.
[c] DB-Wax only.
[d] Unable to resolve compounds **8** and **9**.

The fruits of *P. terebinthus* demonstrated the most diverse and abundant volatile emission, with ten monoterpenes and eight sesquiterpenes in amounts greater than 2%. The female leaves of *P. terebinthus* (Table 1) also showed high diversity in volatile emission, but not to the extent of relatively major amounts of sesquiterpenes. Additionally, the female leaves of *P. lentiscus* (Table 1) showed the highest diversity, albeit low amounts, whereas the fruits of *P. lentiscus* emitted a relatively low number of volatile components and no sesquiterpenes.

Both *P. atlantica* and *P. lentiscus* produced sabinene as the major volatile. Only one investigation of the EO content of buds from male *P. atlantica* reported sabinene as the major constituent;[21] another study lists sabinene as being 'almost constant', albeit not in major amounts, in *P. lentiscus* aerial parts.[19]

The major constituent Δ^3-carene in fruits of *P. chinensis* is an interesting result. The study of the leaves of *P. chinensis* by Zhu *et al.*[18] reported Δ^3-carene as a minor to trace EO component in the plants studied, while two separate reports on *P. atlantica* leaves from Algeria[8,17] give contrasting results for Δ^3-carene amounts. In one investigation[8] the authors report Δ^3-carene as being present in only trace EO amounts in the locations sampled, and in a second investigation[17] the same authors report Δ^3-carene as the major EO constituent in the female leaves from several seasonal samples of *P. atlantica*. The present study showed *P. atlantica* leaves, both male and female, as having no detectable Δ^3-carene in the *ex situ* volatile analysis (Table 1).

Volatile variability

The diverse nature of EO variability of *Pistacia* has been investigated by several research groups. Castola *et al.*[26] surveyed 105 *P. lentiscus* plants for their chemical variability and identified three distinct groups based on the major essential oils, namely terpinen-4-ol/α-pinene, limonene and myrcene. Another key study on *P. lentiscus* performed by Barra *et al.*[19] recognised the fluctuation of the biosynthetic α-terpenyl-cation intermediate and the plant's ability to produce varying EO major constituents based on the seasonal stage of *P. lentiscus*.

This chemical variability exhibited by the numerous *Pistacia* species EO studies brings into question whether the major *ex situ* volatiles can be applied to chemotaxonomic investigation, and thus the inclusion of Fig. 2, which displays several possible routes for the common monoterpenoid carbocation intermediate A. The goal of this paper is not to provide any definitive conclusions regarding chemotypes and/or chemotaxonomy but rather to show that the results do warrant further investigation for chemotype classification. For instance, the data in Table 4 display some very interesting trends in terms of major volatile constituents from fruits of the various *Pistacia* species and/or cultivars. Most notable

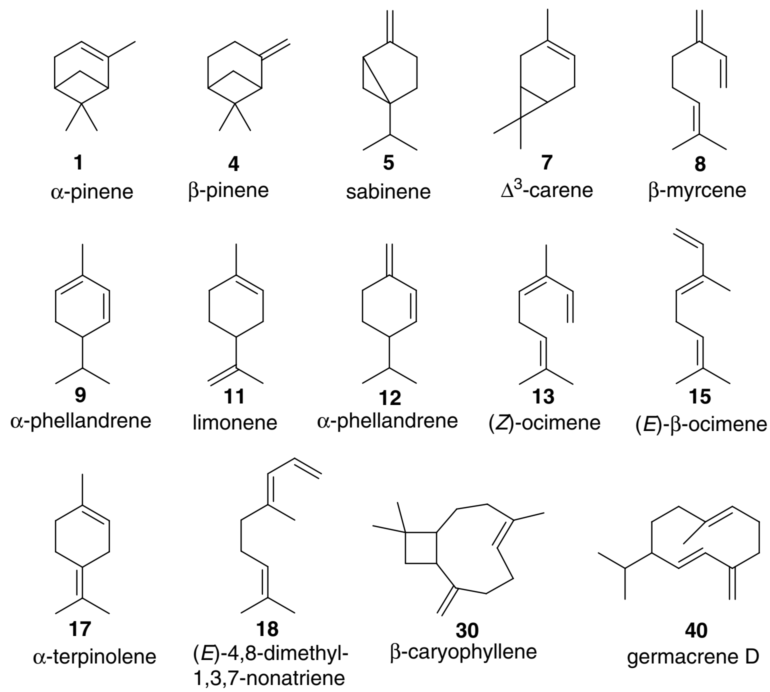

Figure 1. Chemical structures of 14 major volatile components from leaves of female and male *Pistacia* species grown in California. The numbers under the compounds correspond to the numbers in the first column of Table 1.

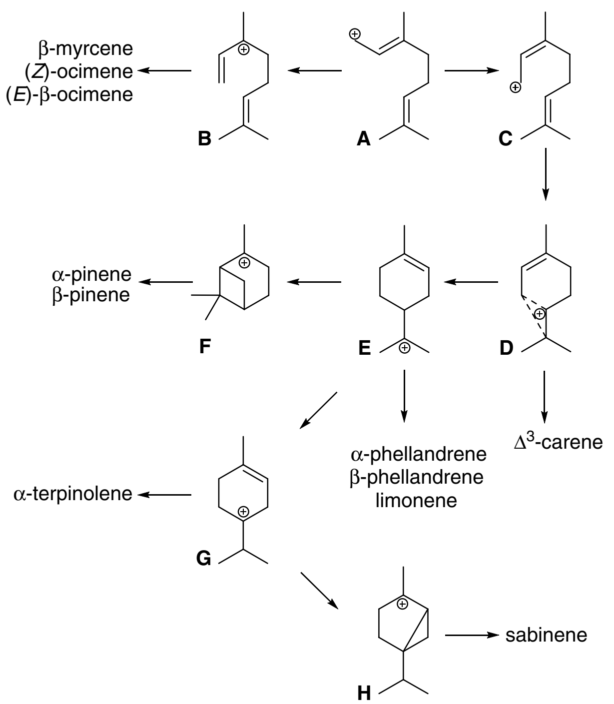

Figure 2. Biosynthetic carbocation precursors of major volatile monoterpenes from *Pistacia* species.

is the consistent emission of limonene, whose production utilises the path of intermediate A to intermediate E in Fig. 2, from the *P. vera* cultivars as well as *P. palaestina* and *P. terebinthus*. Several literature reports of phylogenetic analyses were consulted and an investigation by AL-Saghir[27] revealed some very close associations of *Pistacia* species morphological data with the major volatiles noted in Table 4. Most notable was that the plants with high limonene emissions, *P. palaestina, P. terebinthus* and *P. vera*, were very closely related within branches of the morphological data generated for several trees in the report. The other major compounds, sabinene and Δ^3-carene, were not as closely related to the morphological data as limonene but did show a possible correlation of intermediate H with sabinene on one branch, with a branch split that would give intermediate D to Δ^3-carene (Fig. 2). Similar application of the leaf emissions to the phylogeny trees generated by AL-Saghir[27] also showed some potential correlations for limonene (intermediate E) as well as (*Z*)-ocimene and (*E*)-*β*-ocimene (intermediate B), some of the major *ex situ* volatiles from leaves.

These data suggest that a dendogram based on monoterpene chemotypes,[28] potentially those listed in Table 2, and monoterpene synthases[29] could provide an insight into the genetic differences, and more importantly the possible volatile emissions, of the many *Pistacia* species and cultivars grown in California and central/western Asia.

CONCLUSION

The *ex situ* volatile analysis of the fruits and male and female leaves of several *Pistacia* species and cultivars provided a broad overview of the diverse and rich composition of monoterpene, sesquiterpene, aromatic and green leaf volatiles. The relatively large number of *Pistacia* species allowed not only several comparisons to be performed between *ex situ* volatile samplings, but also for comparison of the emission patterns and relative quantities with the reported EO contents of associated species. Alhough the comparison of *ex situ* emission with EO content did show some corroboration, the relative quantities, particularly the major amounts detected, displayed a large degree of disparity. Ambient volatile analyses of pistachio orchards are currently under way and will help to determine if the differences in terpenoid emissions are geographical in nature or a function of the method used. The major volatile components detected in the analyses provide insight into the chemotaxonomy of *Pistacia* and highlight the need for further investigation into this area of research. The volatiles listed in Table 2 could possibly be used as chemotypes for *Pistacia* species grown in California.

ACKNOWLEDGEMENTS

This research was conducted under USDA-ARS CRIS project 5325-42000-036-00. The authors thank C Simon and H Garrison (National Clonal Germplasm Repository) for their assistance with collections, C Beck for organisational assistance, K. Dragull (USDA) for helpful discussions, and the reviewers for their thoughtful and helpful input.

REFERENCES

1 USDA NASS, *California Fruit & Nut Review* [Online]. (2010). Available: http://www.nass.usda.gov/Statistics_by_State/California/Publications/Fruits_and_Nuts/201002frtrv.pdf [21 July 2010].
2 Kallsen CE, Parfitt DE, Maranto J and Holtz BA, New pistachio varieties show promise for California cultivation. *Calif Agric* **63**:18–23 (2009).
3 Khanazarov AA, Chernova GM, Rakhmonov AM, Nikolyi LV, Ablaeva E, Zaurov DE, *et al*, Genetic resources of *Pistacia vera* L. in Central Asia. *Genet Resour Crop Evol* **56**:429–443 (2009).
4 Food and Agriculture Organization, Food and Agriculture Organization of the United Nations, Productions/Crops statistics. Available: http://faostat.fao.org/site/567/default.aspx [21 July 2010].
5 Duru ME, Cakir A, Kordali S, Zengin H, Harmandar M, Izumi S, *et al*, Chemical composition and antifungal properties of essential oils of three *Pistacia* species. *Fitoterapia* **74**:170–176 (2003).
6 Flamini G, Bader A, Cioni PL, Katbeh-Bader A and Morelli I, Composition of the essential oil of leaves, galls, and ripe and unripe fruits of Jordanian *Pistacia palaestina* Boiss. *J Agric Food Chem* **52**:572–576 (2004).
7 Alma MH, Nitz S, Kollmannsberger H, Digrak M, Efe FT and Yilmaz N, Chemical composition and antimicrobial activity of the essential oils from the gum of Turkish pistachio (*Pistacia vera* L.). *J Agric Food Chem* **52**:3911–3914 (2004).
8 Gourine N, Yousfl M, Bombarda I, Madjemi B, Stocker P and Gaydou EM, Antioxidant activities and chemical composition of essential oil of *Pistacia atlantica* from Algeria. *Ind Crops Prod* **31**:203–208 (2010).
9 Di Donna L, Mazzotti F, Naccarato A, Salerno R, Tagarelli A, Taverna D, *et al*, Secondary metabolites of *Olea europaea* leaves as markers for the discrimination of cultivars and cultivation zones by multivariate analysis. *Food Chem* **121**:492–496 (2010).
10 Winer AM, Arey J, Atkinson R, Aschmann SM, Long WD, Morrison CL, *et al*, Emission rates of organics from vegetation in California's Central Valley. *Atmos Environ* **14**:2647–2659 (1992).
11 Kesselmeier J and Staudt M, Biogenic volatile organic compounds (VOC): an overview on emission, physiology and ecology. *J Atmos Chem* **33**:23–88 (1999).
12 Dragull K, Beck JJ and Merrill GB, Essential oil yield and composition of *Pistacia vera* 'Kerman' fruits, peduncles and leaves grown in California. *J Sci Food Agric* **90**:661–668 (2010).
13 Reddy GVP and Guerrero A, Interactions of insect pheromone and plant semiochemicals. *Trends Plant Sci* **9**:253–261 (2004).
14 Beck JJ, Merrill GB, Higbee BS, Light DM and Gee WS, *In situ* seasonal study of the volatile production of almonds (*Prunus dulcis*) var. 'nonpareil' and relationship to navel orangeworm. *J Agric Food Chem* **57**:3749–3753 (2009).
15 Liu Z, Vidal DM, Syed Z, Ishida Y and Leal WS, Pheromone binding to general odorant-binding proteins from the navel orangeworm. *J Chem Ecol* **36**:787–794 (2010).
16 Beck JJ, Higbee BS, Merrill GB and Roitman JN, Comparison of volatile emissions from undamaged and mechanically damaged almonds. *J Sci Food Agric* **88**:1363–1368 (2008).
17 Gourine N, Yousfl M, Bombarda I, Nadjemi B and Gaydou E, Seasonal variation of chemical composition and antioxidant activity of essential oil from *Pistacia atlantica* Desf. leaves. *J Am Oil Chem Soc* **87**:157–166 (2010).
18 Zhu B, Wang Q, Roge EF, Nan P, Liu Z and Zhong Y, Chemical variation in leaf oils of *Pistacia chinensis* from five locations in China. *Chem Nat Prod* **42**:422–425 (2006).
19 Barra A, Coroneo V, Dessi S, Cabras P and Angioni A, Characterization of the volatile constituents in the essential oil of *Pistacia lentiscus* L. from different origins and its antfungal and antioxidant activity. *J Agric Food Chem* **55**:7093–7098 (2007).
20 Ozcan MM, Tzakou O and Couladis M, Essential oil composition of the turpentine tree (*Pistacia terebinthus* L.) fruits growing wild in Turkey. *Food Chem* **114**:282–285 (2009).
21 Tzakou O, Bazos I and Yannitsaros A, Volatile metabolites of *Pistacia atlantica* Desf. from Greece. *Flav Fragr J* **22**:358–362 (2007).
22 Chryssavgi G, Vassiliki P, Athanasios M, Kibouris T and Michael K, Essential oil composition of *Pistacia lentiscus* L. and *Myrtus communis* L.: evaluation of antioxidant capacity of methanolic extracts. *Food Chem* **107**:1120–1130 (2008).
23 El-Sayed AM, *The Pherobase: Database of Insect Pheromones and Semiochemicals*. [Online]. Available: http://www.pherobase.com [26 July 2010].
24 Pinero JC and Dorn S, Synergism between aromatic compounds and green leaf volatiles derived from the host plant underlies female attraction of the oriental fruit moth. *Entomol Exp Appl* **125**:185–194 (2010).
25 Chahed T, Dhifi W, Hosni K, Msaada K, Kchouk ME and Marzouk B, Composition of Tunisian pistachio hull essential oil during fruit formation and ripening. *J Essent Oil Res* **20**:122–125 (2008).
26 Castola V, Bighelli A and Casanova J, Instraspecific chemical variability of the essential oil of *Pistacia lentiscus* L. from Corsica. *Biochem Syst Ecol* **28**:79–88 (2000).
27 AL-Saghir MG, Phylogenetic analysis of the genus *Pistacia* (Anacardiaceae). *PhD Dissertation*, Virginia Polytechnic Institute and State University, Blacksburg, VA, pp. 27–29 (2006).
28 Roussis V, Petrakis PV, Ortiz A and Mazomenos BE, Volatile constitutents of needles of five *Pinus* speces grown in Greece. *Phytochemistry* **39**:357–361 (1995).
29 Wise ML, Savage TJ, Katahira E and Croteau R, Monoterpene synthases from common sage (*Salvia officinalis*). *J Biol Chem* **273**:14891–14899 (1998).